EXHIBIT 1, Tab 16

CONCISE COMMUNICATIONS

Counterpoint from the trenches: a pragmatic approach to therapeutic trials in rheumatoid arthritis

In response to the recent editorial by Dr. Boers (1), I would like to offer comments from another perspective. It is not so much that clinical trialists are unaware of the disadvantages of the add-on or step-up trial design as that we are cognizant of ethical issues that restrict the use of placebo. The add-on or step-up design allows a placebo comparison and should continue to be used, but not in large trials supporting regulatory approval. This design is useful early in a clinical development program, provided attention is paid to the enrollment criteria that define "failed" background therapy.

It is methodologically elegant to recommend that, in the presence of persistently active disease despite an adequate trial of a disease-modifying antirheumatic drug (DMARD) such as methotrexate (MTX), patients should stop receiving their prior therapy (washout period) and then be randomized to receive active or placebo therapy with rescue medication at 3 months. However, practical considerations limit the feasibility of this approach. With the new therapeutic options available to patients, it is unclear how many would consent to a washout period, which typically lasts 4 weeks, when they may be at risk for disease flares. Furthermore, it is unlikely that this design would be popular with ethics review boards.

Ethically, before an agent is used as a stand-alone therapy, for 3 or 6 months, it must be shown that it offers clinical benefit. Currently, that is possibly only in a background therapy situation, despite the potential difficulties of drug–drug and mechanism-of-action interactions. Once an experimental treatment has been shown to improve clinical parameters, even when superimposed on a failed therapy, then it can be introduced into clinical trials as monotherapy, such as the 3-arm randomized controlled trial (RCT) advocated by Dr. Boers.

Although a "true" signal of efficacy can more easily be determined against placebo than against active treatment, the present state of therapy suggests that use of a true placebo is neither ethical nor appropriate. Withholding active treatment for 4–6 months has been shown to result in losses of physical function that are not regained when active treatment is instituted. In a placebo-controlled study comparing leflunomide with MTX (2), patients receiving placebo entered alternate therapy with leflunomide on or after month 4, for documented lack of efficacy. Despite similar baseline modified Health Assessment Questionnaire (HAQ) (3) scores, end point scores were 0.75 and 0.50, respectively, in the active-treatment and placebo groups after 12 months. Following 12 months of active treatment, American College of Rheumatology 20% (ACR20) responses (4) were 52% in both groups; less improvement in physical function reflected 4 months of treatment with placebo (5).

Similarly, in an RCT examining combination treatment with leflunomide added to failed MTX therapy, patients who received placebo MTX for 0–6 months were switched to leflunomide plus MTX for 6–12 months (6). The mean improvement in the HAQ disability index (from baseline to 12 months) was −0.54 in patients who received combination therapy for the entire period, compared with −0.30 in those receiving combination therapy only during the second 6-month

period. Despite ACR20 responses of 56% and 58%, respectively, and similar improvements in the Medical Outcomes Survey Short Form 36 (SF-36), following 6 months of combination therapy, the differences in mean improvements in the HAQ disability index reflected 6 months of treatment with placebo (7).

Dr. Boers also highlighted a critical methodologic issue with background therapy trials: the term "partical responders" can refer to a variety of patient populations. Rather than precluding use of this trial design, instead results should be interpreted more precisely according to the inclusion criteria of the RCT. Patients with active RA may be enrolled because they 1) failed MTX therapy after having an initial response, 2) never responded to or could not tolerate MTX, 3) had received treatment for a limited time such that a maximal response to MTX had not yet occurred, or 4) had a response to MTX that was not considered adequate by the enrolling investigator. Each of these has different implications and leads to the possibility of recruiting a diverse group of patients.

When comparing data, it is important to know the required duration of prior background therapy and the minimum and maximum doses that were received. Placebo- and active-drug–controlled trials have established that the time to maximal response with MTX therapy is ~6 months, although most patients show evidence of improvement at 3 months. If patients fail to improve after receiving MTX for 3 months, is it ethical for physicians to ask these patients to enroll in an RCT in which they would continue a "failed" treatment and might receive placebo for 3 or 6 months? It is also possible that a patient who experiences little enough improvement after 3 months of MTX therapy could enroll in a step-up trial, receive placebo, and then demonstrate further improvement with background therapy, thereby confounding results in the placebo-treatment arm. Presumably, such an occurrence is infrequent but may explain the higher response rates with placebo that are generally observed in RCTs involving background therapy.

Practice has driven the use of background MTX therapy plus biologic agents, but it is unclear whether this approach offers benefit by decreasing potential immunogenicity (as stated by Dr. Boers) or altering clearance of the agent. Pharmacokinetic data for several biologic DMARDs indicate that concomitant administration of MTX prolongs the half-life of the biologic agent but does not appreciably alter the maximal concentration levels (8,9). This is an important point when evaluating combination therapy with synthetic or biologic agents, because potential pharmacokinetic interactions may influence the results.

For example, in an RCT of cyclosporine plus "failed" MTX (10,11), patients who received placebo plus MTX for 6 months, were switched to MTX plus cyclosporine for the next 6 months. In contrast to patients who received combination therapy over the entire 12-month period (whose ACR20 responses at 6 and 12 months were 48% and 54%, respectively), ACR20 responses increased from 16% at 6 months to only 20% at 12 months in those who received MTX plus cyclosporine only during the second 6-month period. These data suggest that adding cyclosporine to MTX increased serum concentrations of MTX without offering additional clinical benefit. Because it was required that these patients had been receiving MTX for ≤3 months before enrollment, some were

CONCISE COMMUNICATIONS

Table 1. Combination-therapy randomized controlled trials with background MTX*

	MTX + placebo/CsA	MTX + placebo/ETN	ATTRACT: MTX + placebo/IFN	MTX + placebo/IL-1Ra	MTX + placebo/LEF
Year (ref.)	1995–1997 (10,11)	1999 (15)	2000 (16,17)	2002 (18)	2002 (6,19)
Total no. of patients (no. per group)	148 (75/73)	89 (30/59)	428 (88/340)†	398 (48/269)‡	263 (133/130)
Disease duration, mean years	9.4/11.2	13/13	11/10.4	7.8/7.4	12.7/10.5
Mean no. of DMARDs failed	1.8/1.8	2.8/2.7	3/3	2.1/1.8	1.04/1.05
Required duration of prior MTX therapy (dosage)	≥3 mos (≤15 mg/wk)	≥6 mos (≥15 mg/wk)	≥6 mos (≥12.5 mg/wk)	≥6 mos (15–25 mg/wk)	≥6 mos (15–20 mg/wk)
Years reported	NR/NR	2.9/4.8	3/3	NR/NR	2.2/2.4
Mean failed dose	NR/NR	18 mg/19 mg	16 mg/16–17 mg	16.3 mg/17 mg	16.1 mg/16.8 mg
Baseline characteristic					
Tender joint count, mean	20/19	28/28	31/31–34	28/24	26/27
Swollen joint count, mean	17/15	20/21	21/21–24	18/18	19/17
Modified HAQ disability index, mean	1.4/1.4	1.3/1.49	1.7/1.7–1.8	1.4/1.4	1.5/1.6
DAS28, median	–	–	–	–	6.1/6.0
Trial duration	6 months	6 months§	24 months	6 months	6 months
ACR20 at 6 months, % (ITT, LOCF)	16/48	27/71	20/50–58	23/42	23/52
DAS28 good or moderate Δ, %	–	–	27/58–67	–	37/67
Followup (duration, design)	6 months, open-label	–	12 months, blinded	–	6-months, open-label
No. of patients	44/48	–	88/81–87	–	95/96
DAS28 at 6 months	–	–	–	–	5.5/4.5
ACR20 6→12 months, % (ITT, LOCF)	16→20/48→54	–	17/42–59	–	27→58/59→56
DAS28 good or moderate Δ, %	–	–	23/57–70	–	78/73
DAS28 at 12 months	–	–	–	–	4.2/4.1
SF-36 administered	No	No	Yes	No	Yes

* MTX = methotrexate; ATTRACT = Anti–Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy; CsA = cyclosporin A; ETN = etanercept; IFN = interferon; IL-1Ra = interleukin-1 receptor antagonist; LEF = leflunomide; DMARDs = disease-modifying antirheumatic drugs; NR = not reported; HAQ = Health Assessment Questionnaire; ACR20 = American College of Rheumatology criteria for 20% improvement; ITT = intent-to-treat; LOCF = last observation carried forward; DAS28 = disease activity score in 28 joints; SF-36 = Medical Outcomes Survey Short Form 36.
† Four dosages of anti–tumor necrosis factor α monoclonal antibody, 81–87 patients per dosage group.
‡ Five dosages of IL-1Ra, 46–63 patients per dosage group. For comparability, 24-week data are presented.
§ Primary end point at 3 months.

1346

CONCISE COMMUNICATIONS

Table 2. Combination-therapy randomized controlled trials with background MTX and/or DMARDs with rescue*

	DE 009 MTX + placebo/ADA	DE 019 MTX + placebo/ADA	DE 031 DMARDs + placebo/ADA	MTX + placebo/IL-1Ra	DMARDs + placebo/IL1-Ra
Year (ref.)	2003 (20,21)	2003 (21,22)	2003 (21,23)	2001 (24,25)	2003 (26)
Total no. of patients (no. per group)	271 (62/209)†	619 (200/419)	636 (318/318)	906 (453/453)‡	1,399 (283/1,116)
Disease duration, mean years	11.1/12.2	10.9/11	11.5/9.3	9.9/10.7	10.7/10.2
Mean no. of DMARDs failed	2.9/3	2.4/2.4	2.2/2.2	NR/NR	NR
Required duration of prior MTX therapy (dosage)	≥3 mos (≥12.5–15 mg/wk)	≥3 mos (≥12.5–15 mg/wk)	NA	≥6 mos (10–25 mg/wk)	NA
Mean failed dose	16.5 mg/16.4 mg	16.7 mg/16.7 mg	–	15.8 mg/15.7 mg	–
Baseline characteristic					
Tender joint count, mean	29/29	28/28	28/27	26/26	23/23
Swollen joint count, mean	17/17	19/19	21/21	20/20	18/19
HAQ disability index, mean	1.6/1.5	1.5/1.4	1.4/1.4	1.4/1.4	NR/NR
DAS28, median	5.7/5.7	5.6/5.6	5.7/5.7	6.3/6.3	NR/NR
Trial duration	6 months	12 months	6 months	6 months	6 months
Rescue medication administered	≥8 weeks	≥8 weeks	≥8 weeks	≥16 weeks	Dose changes allowed
ACR20 at 6 months, % (ITT, LOCF)	13/67	30/63	35/53	22/38	NR
DAS28 good or moderate Δ, %	NR/72	NR/72	NR/71	NR/NR	NR
Followup (duration, design)	–	Blinded continuation	–	–	24 months, open-label
No. of patients	–	140/160	–	–	–
DAS28 at 6 months	5.5/2.7	5.3/3.4	5.1/3.8	5.2/4.7	NR/NR
ACR20 6→12 months, % (ITT, LOCF)	–	24/59	–	NR/NR	NR/NR
DAS28 at 12 months	–	NR/NR	–	5.3/4.6	NR/NR
SF-36 administered	Yes	Yes	Yes	Yes	Yes

* MTX = methotrexate; DMARDs = disease-modifying antirheumatic drugs; ADA = adalimumab; IL-1Ra = interleukin-1 receptor antagonist; NR = not reported; NA = not applicable; HAQ = Health Assessment Questionnaire; DAS28 = disease activity score in 28 joints; ACR20 = American College of Rheumatology criteria for 20% improvement; ITT = intent-to-treat; LOCF = last observation carried forward; SF-36 = Medical Outcomes Survey Short Form 36.
† Three doses of ADA, 67–73 patients per dose group.
‡ For the interim analysis, n = 253 placebo subjects and n = 253 IL-1Ra subjects.

CONCISE COMMUNICATIONS

still attaining maximal responses that may have been enhanced by an increase in MTX bioavailability (12).

Therefore, to better understand whether add-on treatment is beneficial, it is important to confirm that the responses that are achieved once the placebo group receives active treatment (e.g., over 6–12 months after 0–6 months on placebo) are at least similar to responses at 6 months in patients initially receiving active treatment, and that beneficial responses following 0–6 months of active treatment are maintained over 6–12 months. To date, only 2 such follow-on trials have been reported—one that was confirmatory and one that was not (6,11).

As reviewed in Tables 1 and 2, RCTs in patients receiving background therapy with MTX have been performed with all of the recently approved DMARDs. The study populations were similar: patients with long disease duration having failed treatment with multiple DMARDs. The mean dosage of failed MTX therapy was remarkably similar across trials: 16–19 mg/week, for a mean duration of 2–4 years, when reported. Inclusion criteria for the more recent RCTs required only 3 months of prior treatment with MTX, with rescue therapy allowed as early as week 8 (Table 2). Judging by the tender and swollen joint counts and the HAQ disability index scores at baseline, patients in these trials had active disease, as confirmed by baseline Disease Activity Scores (DAS28) (13) reported in 5 RCTs. In general, responses according to ACR20 and European League Against Rheumatism criteria (14) were comparable, although responses were somewhat higher by the DAS28. Use of both ACR response criteria and DAS28 scores facilitates comparison of responses and baseline disease activity across trials, and both should be reported. The time to institution of rescue therapy must also be carefully considered, so that an adequate comparator group is available to confirm efficacy.

In summary, given that step-up trials may best initially qualify a promising new therapy for further clinical evaluation, practical implications require more carefully defined inclusion criteria for RCTs involving background therapy. These must be clearly specified in publications to permit better understanding of the role failed therapy may have played in efficacy results. Second, the academically pure design recommended by Dr. Boers is problematic due to ethical considerations. Third, based on results from successful step-up trials, 3-arm RCTs currently underway of either DMARD alone versus the combination should help us to understand whether combination therapy offers true synergy. Preliminary data in patients with early RA suggest this to be true, which is an exciting development. Finally, however helpful these trials have been for approving new therapies, no data from RCTs currently help us understand, in the case of failed therapy, whether patients should receive additional treatment or switch therapies.

The issues raised by Dr. Boers are cogent and require attention. I hope that my comments will continue the discussion by reinforcing the key points of that editorial, and by suggesting pragmatic approaches to addressing those concerns.

Vibeke Strand, MD
Stanford University
Portola Valley, CA

1. Boers M. Add-on or step-up trials for new drug development in rheumatoid arthritis: a new standard? Arthritis Rheum 2003;48: 1481–3.
2. Strand V, Cohen S, Schiff M, Weaver A, Fleischmann R, Cannon F, et al, for the Leflunomide Rheumatoid Arthritis Investigators Group. Treatment of active rheumatoid arthritis with leflunomide compared with placebo and methotrexate. Arch Intern Med 1999;159:2542–50.
3. Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. Arthritis Rheum 1980;23:37–45.
4. Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, et al. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995;38:727–35.
5. Olsen N, Schiff M, Strand V. Alternate therapy with leflunomide (LEF) or methotrexate (MTX) after switch from initial treatment in patients with active rheumatoid arthritis (RA) [abstract]. Arthritis Rheum 1999;42 Suppl 9:S241.
6. Kremer JM, Genovese MC, Cannon GW, Caldwell J, Cush J, Furst DE, et al. Combination leflunomide and methotrexate therapy for patients with active rheumatoid arthritis failing methotrexate monotherapy: an open-label extension of a randomized double-blind, placebo-controlled trial. Submitted for publication.
7. Ware JE Jr, Snow KK, Kosinski M, Gandek B. SF-36 health survey: manual and interpretation guide. Boston: The Health Institute, New England Medical Center; 1993.
8. St Clair EW, Wagner CL, Fasanmade AA, Wang B, Schaible T, Kavanaugh A, et al. The relationship of serum infliximab concentrations to clinical improvement in rheumatoid arthritis: results from ATTRACT, a multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2002;46:1451–9.
9. Package insert. Humira (adalimumab). Abbott Park (IL): Abbott; 2002.
10. Tugwell P, Pincus T, Yocum D, Stein M, Gluck O, Kraag G, et al, and the Methotrexate-Cyclosporine Combination Study Group. Combination therapy with cyclosporine and methotrexate in severe rheumatoid arthritis. N Engl J Med 1995;333:137–41.
11. Stein CM, Pincus T, Yocum D, Tugwell P, Wells G, Gluck O, et al, and the Methotrexate-Cyclosporine Combination Study Group. Combination treatment of severe rheumatoid arthritis with cyclosporine and methotrexate for forty-eight weeks: an open-label extension study. Arthritis Rheum 1997;40:1843–51.
12. Package insert. Sandimmune (cyclosporine). East Hanover (NJ): Novartis; 2002.
13. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight–joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8.
14. Van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis: comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism criteria. Arthritis Rheum 1996;39:34–40.
15. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, et al. A trial of etanercept, a recombinant tumor necrosis factor receptor: Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med 1999; 340:253–9.
16. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. N Engl J Med 2000;343:1594–602.
17. Antoni CE, Kavanaugh A, Manger B, Keenan G, Schiable T, Harriman G. Responses to infliximab therapy in the ATTRACT

trial assessed with the Disease Activity Score (DAS) [abstract]. Ann Rheum Dis 2001;60 Suppl:S170.

18. Cohen S, Hurd E, Cush J, Schiff M, Weinblatt ME, Moreland LW, et al. Treatment of rheumatoid arthritis with anakinra, a recombinant human interleukin-1 receptor antagonist, in combination with methotrexate: results of a twenty-four–week, multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2002;46:614–24.

19. Kremer JM, Genovese MC, Cannon GW, Caldwell JR, Cush JJ, Furst DE, et al. Concomitant leflunomide therapy in patients with active rheumatoid arthritis despite stable doses of methotrexate: a randomized, double-blind, placebo-controlled trial. Ann Intern Med 2002;137:726–33.

20. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, et al. Adalimumab, a fully human anti-tumor necrosis factor α monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial [published erratum appears in Arthritis Rheum 2003;48:855]. Arthritis Rheum 2003;48:35–45.

21. Schiff M, Furst D, Kavanaugh AF, van Riel PL, Fischkoff S, Chartash E, et al. Efficacy of adalimumab measured by the Disease Activity Score 28 (DAS28) and EULAR response criteria [abstract]. Ann Rheum Dis 2003;62 Suppl 2:S170.

22. Keystone E, Kavanaugh AF, Sharp J, Hua Y, Teoh L, Fischkoff S, et al. Adalimumab (D2E7), a fully human anti-TNF-α monoclonal antibody, inhibits the progression of structural joint damage in patients with active RA despite concomitant methotrexate therapy [abstract]. Arthritis Rheum 2002;46 Suppl 9:S205.

23. Furst DE, Schiff MH, Fleischmann RM, Strand V, Birbara CA, Compagnone D, et al. Adalimumab, a fully human antitumor necrosis factor-α monoclonal antibody, and concomitant standard antirheumatic therapy for the treatment of rheumatoid arthritis: results of STAR (Safety Trial of Adalimumab in Rheumatoid Arthritis). J Rheumatol. 2003;30:2563–71.

24. Cohen SB, Moreland LW, Cush JJ, Greenwald MW, Block JA, Shergy WJ, et al. Anakinra (recombinant interleukin-1 receptor antagonist): a large, placebo controlled efficacy trial of anakinra in patients with erosive rheumatoid arthritis disease [abstract]. Arthritis Rheum 2001;44:2946.

25. Shergy WJ, Kaine JL, Hurd ER, Cohen SB, Roth S, Brodsky A, et al. Disease Activity Score in a large placebo controlled randomized study of rheumatoid arthritis patients treated with anakinra [abstract]. Ann Rheum Dis 2003;62 Suppl 2:S186.

26. Fleischmann RM, Schechtman J, Bennett R, Handel ML, Burmester G-R, et al. Anakinra, a recombinant human interleukin-1 receptor antagonist (r-metHuIL-1ra), in patients with rheumatoid arthritis: a large, international, multicenter, placebo-controlled trial. Arthritis Rheum 2003;48:927–34.

Polymorphisms of the Fcγ receptor type IIB gene are not associated with systemic lupus erythematosus in the Swedish population

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease with a wide spectrum of clinical features. There is strong evidence for a genetic component in the etiology of SLE, although the mode of inheritance seems to be complex. The genetic contribution entails several different polymorphic genes rather than rare mutations with high penetrance. The presence of genetic heterogeneity is extended, which means that there will be different susceptibility genes depending on the ethnicity and geographic location of the population studied (1).

Several polymorphisms of the different low-affinity Fcγ receptor (FcγR) genes have been shown to alter the ligand specificity and the effector functions mediated through the Fcγ receptors. In fact, these polymorphisms have been associated with an increased risk for development of SLE (2,3). Recently, a single-nucleotide polymorphism (SNP) in exon 5 of the FcγR2B gene causing a nonsynonymous amino acid change in the transmembrane region (FcγR2B-232I/T) was reported to be associated with SLE in a Japanese population (4). This is a strong candidate gene due to the inhibitory function of FcγR2B, which acts through an immunoreceptor tyrosine-based inhibitory motif (ITIM) embedded in the cytoplasmic domain. Furthermore, certain mouse strains deficient for FcγR2b have been shown to develop lupus-like conditions similar to the human disease (5). The change at nucleotide c.695 (T>C) was the first reported SNP in the FcγR2B gene, and since it changes an amino acid in the transmembrane region, it might have a physiologic effect on the receptor function, although this remains to be investigated. To date, an association between this gene variant and SLE has not been reported in other populations.

In the present study we investigated the role of FcγRIIB in SLE susceptibility. We performed genotyping of the FcγR2B-232I/T SNP and an additional novel SNP in the 3'-untranslated region (FcγR2B.3-A/G) 193 bp downstream of the stop codon. The FcγR2B.3 SNP was identified by a computer-based bioinformatics search in the dbSNP (www.ncbi.nlm.nih.gov/; reference SNP Id: rs12721) and verified to be polymorphic by direct sequencing in 16 control subjects. We conducted a case–control study with 263 female SLE cases and 228 female healthy controls. Patients and controls were all of Swedish ancestry (ethnic background was determined based on the ancestries of the great-grandparents, using a questionnaire). All patients were diagnosed by a specialist and fulfilled the American College of Rheumatology 1982 revised criteria for the classification of SLE (6). Because of the overrepresentation of women with SLE (9:1), only female patients and controls were included in the analysis.

Fcγ2B.3 was genotyped using pyrosequencing (7) with the outer primers 5'-GGGAGATGCTGCAGTTCCAAAA-GA-3' (sense) and 5'-CTAACCTGTAACATAAGCATTT-CCCA-3' (antisense), followed by amplification with the inner primers 5'-CTTCCAGAGTCATCTACCTGAGTC-3' (sense) and 5'-GGATGTGGAACGGAAGAGCCTTG-3'. The FcγR2B-232I/T was amplified using nested polymerase chain reaction (PCR) with the outer primers Ex8F (5'-GGTGCATGAGAAGTGAATAGGTGA-3') and Ex5R (5'-GAAGCAGAGCTCCCTCGTTG-3'). The inner PCR procedure was done using the Ex5R primer and the Ex5F primer (5'-CAATACGGGCCTAGATCTGAATGT-3'), and then the fragment was sequenced using the ex5F primer (Figure 1). Primers were designed so that amplification of FcγRIIC would be avoided.

The alleles and genotypes for the 2 FcγRIIB gene variants were tested for association (Table 1). The distribution of alleles and genotypes was in Hardy-Weinberg equilibrium both in cases and in controls, and there was no significant difference in genotype or allele frequencies between cases and controls. Thus, in contrast to the results in the Japanese study

CONCISE COMMUNICATIONS

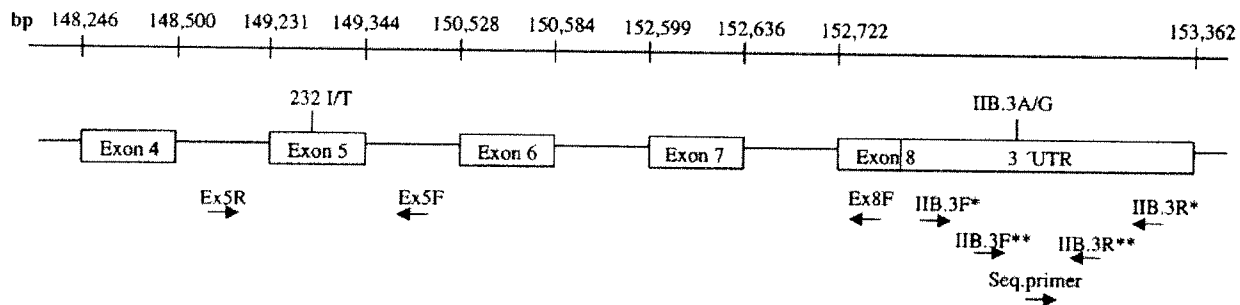Homo Sapiens chromosome 1 genomic contig: NT_004668

Figure 1. Schematic representation of the structure of the *FcγR2B* gene according to the Human Genome Sequence in ensembl (www.ensembl.org). The locations of the primers for amplification of the I>T polymorphism and the IIB.3 polymorphism are shown. Outside primers and nested inside primers for *FcγR2B.3* are indicated by single asterisks and double asterisks, respectively. 3'-UTR = 3'-untranslated region.

(4), we did not find association between SLE and the *FcγR2B*-232I/T polymorphism. *FcγR2B.3* also showed no association.

The discrepancy between our findings and those reported by the Japanese group could have the following explanations. First, the impact on disease susceptibility may differ between populations and the *FcγR2B*-232I/T might be a susceptibility factor only in populations of certain descent. In this respect, there was a significant difference in frequencies between the Japanese and the Swedish control groups, but there was no difference between Swedish controls in the present study and another group of control subjects from the Netherlands (4). This shows that the genetic history of the

FcγR2B-232I/T gene variant differs in these populations. Association analysis with a Dutch lupus patient population was not reported by the Japanese authors (4).

Second, even if genetic heterogeneity exists and factors such as genetic drift and population migration may explain the conflicting results, the association found in the initial study was only marginally significant and could be due to a Type I error (false-positive result). The positive association needs therefore to be replicated in an independent study before the *FcγR2B*-232T allele can be considered to be a true risk factor for SLE. However, we cannot rule out the possibility that polymorphisms in the *FcγR2B* gene are of minimal importance in Caucasian populations but of major importance in Asian populations.

The *FcγR2B* gene has been suggested as a candidate SLE susceptibility gene partly because of its importance in determining the cell response to immunologic stimuli and in maintaining peripheral tolerance. Other genes that, like *FcγR2B*, carry ITIM motifs, such as *CD22* and *PDCD1*, have been suspected to be involved in SLE because of their essential role in regulating the immune response and preventing autoimmunity. For example, the *PDCD1* gene was recently identified as a susceptibility factor for SLE in several populations (8).

The importance of FcγRIIB is also evidenced by the findings of several studies in mice, in which certain strains deficient for this gene display enhanced responses to antibody/antigen immune complexes as well as a breakdown in tolerance, leading to development of autoimmunity (5,9). However, since this effect is observed only in certain mouse strains, the *Fcγ R2b* gene may act as a genetic susceptibility factor for SLE only in the presence of epistatic modifiers found in the presence of specific genetic backgrounds.

In conclusion, we did not find any evidence of association between *FcγR2B* and SLE susceptibility in the Swedish population. In order to evaluate the disease contribution of this gene variant and other variants of the *FcγR2B* gene in the development of SLE, further investigations must be conducted in different populations.

Table 1. Distribution of alleles and genotypes of the Fcγ receptor type IIB (FcγRIIB) polymorphisms in Swedish patients and controls*

SNP, genotype	Controls, no. (%)	SLE cases, no. (%)
FcγR2B-232I/T genotype		
I/I	171 (75)	189 (72)
I/T	53 (23)	67 (25)
T/T	4 (2)	7 (3)
FcγR2B.3-A/G genotype		
G/G	81 (44)	107 (45)
G/A	84 (46)	98 (41)
A/A	19 (10)	31 (13)
FcγR2B-232I/T allele		
I	395 (87)	445 (85)
T	61 (13)	81 (15)
FcγR2B.3-A/G allele		
G	246 (67)	312 (66)
A	122 (33)	160 (34)

* Association was tested by chi-square analyses using 2 × 2 contingency tables. *P* values were calculated using Fisher's exact test. The analysis was performed assuming dominant or recessive modes of inheritance for each locus. Allele frequencies were calculated from the sample set used. Both single-nucleotide polymorphisms (SNPs) were found to be in Hardy-Weinberg equilibrium. The numbers of individuals genotyped for each SNP were as follows: for the *FcγR2B*-232I/T SNP 228 controls and 263 SLE patients, for the *FcγR2B.3*-A/G SNP 184 controls and 236 SLE patients.

V. Magnusson, PhD
University of Uppsala
Uppsala, Sweden
R. Zunec, PhD
University of Uppsala
Uppsala, Sweden
and Clinical Hospital Zagreb
Zagreb, Croatia
J. Odeberg, MD, PhD
Royal Institute of Technology
Stockholm, Sweden
G. Sturfelt, MD, PhD
L. Truedsson, MD, PhD
Lund University Hospital
Lund, Sweden
I. Gunnarsson, MD, PhD
Karolinska Institute
Stockholm, Sweden
M. E. Alarcón-Riquelme, MD, PhD
University of Uppsala
Uppsala, Sweden

1. Johanneson B, Lima G, von Salome J, Alarcon-Segovia D, Alarcon-Riquelme ME, Collaborative Group on the Genetics of SLE, The BIOMED II Collaboration on the Genetics of SLE and Sjogrens syndrome. A major susceptibility locus for systemic lupus erythemathosus maps to chromosome 1q31. Am J Hum Genet 2002;71: 1060–71.
2. Salmon JE, Millard S, Schachter LA, Arnett FC, Ginzler EM, Gourley MF, et al. Fc gamma RIIA alleles are heritable risk factors for lupus nephritis in African Americans. J Clin Invest 1996;97: 1348–54.
3. Koene HR, Kleijer M, Swaak AJG, Sullivan KE, Bijl M, Petri ME, et al. The Fc γ RIIIA-158F allele is a risk factor for systemic lupus erythematosus. Arthritis Rheum 1998;41:1813–8.
4. Kyogoku C, Dijstelbloem HM, Tsuchiya N, Hatta Y, Kato H, Yamaguchi A, et al. Fcγ receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus: contribution of FCGR2B to genetic susceptibility. Arthritis Rheum 2002;46: 1242–54.
5. Bolland S, Ravetch JV. Spontaneous autoimmune disease in Fc-(gamma)RIIB-deficient mice results from strain-specific epistasis. Immunity 2000;13:277–85.
6. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
7. Ronaghi M, Uhlen M, Nyren P. A sequencing method based on real-time pyrophosphate. Science 1998;281:363–5.
8. Prokunina L, Castillejo-Lopez C, Oberg F, Gunnarsson I, Berg L, Magnusson V, et al. A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. Nat Genet 2002;32:666–9.
9. Wernersson S, Karlsson MC, Dahlstrom J, Mattsson R, Verbeek JS, Heyman B. IgG-mediated enhancement of antibody responses is low in Fc receptor gamma chain-deficient mice and increased in Fc gamma RII-deficient mice. J Immunol 1999;163:618–22.

Erratum

In the concise communication by Redlich et al published in the March 2004 issue of *Arthritis & Rheumatism* (pp 1001–1005), Figures 1 and 2 were inadvertently transposed at a late stage in the production, such that the correct legend for Figure 1 appears on page 1002 but with the illustration of Figure 2, and the correct legend for Figure 2 appears on page 1004 but with the illustration of Figure 1. The correct Figures 1 and 2 with their matching legends may be viewed at www.interscience.wiley.com/jpages/0004-3591/suppmat/index.html.

We regret the error.